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Physicochemical and Cytological Analysis of Synovial Fluid in Young and Adult Dogs

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ABSTRACT

Background: Synovial fluid is a complex viscous fluid which provides lubrication to the articulating joints to avoid friction and wearing of cartilages. It is also an indicator of health status of joint, but a comparison of its composition between young and adult dogs is lacking.

Methods: Present study was conducted to evaluate physical, biochemical and cytological analysis of synovial fluid in apparently healthy stifle joint of young and adult non-descript dogs. Twelve non-descript dogs irrespective of sex were divided into two groups (6 dogs in each group) *i.e.,* group I (2-8 months of age) and group II (1-8 years of age). Dogs died in the OPDs of Veterinary Clinical Complex, Jabalpur were acquired with the consent of pet parent. The duration of study was one year, *i.e.,* from September 2021 to August 2022. Immediately after collection of synovial fluid; volume, colour, viscosity, pH, specific gravity and refractive index were recorded, smear on slides were prepared. Protein and glucose were estimated within one hour using biochemical semi-autoanalyzer.

Result: Volume of fluid collected in a single attempt varied from one drop to 0.5 ml. Mean pH values in group I and II were 7.33±0.17 and 7.67±0.25 respectively. Mean values of refractive index and specific gravity were 1.34 and 1.02 respectively in both the groups. Observations of mucin clot test of synovial fluid in group I and II exhibited a tight ropy clot in a clear solution. Turbidity was not observed in any case. Mean protein concentration was 1.29±0.18 g/dl and 2.74±0.84 g/dl in group I and II respectively. The concentration of glucose in group I and II was 102.79±3.66 mg/dl and 105.47±4.19 mg/dl respectively, that was around normal blood plasma level. Cytological observation revealed 1-3 cells per field at 1000x magnification. Mean neutrophil count in both the groups was about 6% of total nucleated cell count. Small mononuclear cells and large mononuclear cells were 19±3.79, 74.33±4.24 and 12.17±2.33, 81.83±2.54 in group I and II respectively. Biochemically and cytologically no significant difference was observed between the group I and II. Vacuolated large mononuclear cells contributed about 6-6.5% of total large mononuclear cells in both the groups. No significant difference was observed in most physical and biochemical parameters of synovial fluid in young and adult dogs, except a higher concentration of protein content in adults.

Key words: Biochemical, Cytological, Dog, Physical, Synovial fluid

INTRODUCTION

Every move depends on healthy joints that work smoothly and are essential for quality life in dogs. The intricate structure of a joint is remarkable, and maintaining the health of joint components is key to long-term mobility and joint functioning. Synovial joints are those in which the articulating bones are separated by a fluid-containing joint cavity bounded by joint capsule. Synovial fluid is a complex viscous fluid which provides lubrication to the articulating joints to avoid friction and wearing of cartilages (Smith, 2011). This arrangement permits substantial freedom of movement.

Synovial fluid is considered as dialysate of plasma modified by constituents secreted by synovial membrane. The major difference between synovial fluid and other body transudate derived from plasma is the high content of hyaluronic acid (mucin) which maintains its normal viscosity. Besides, it helps in the nutrition of articular cartilage by acting as a transport medium for nutritional substances, such as glucose, and aid in the mechanical function of lubrication over the articulating surfaces (Cornelius, 1963). Arthrocentesis has both diagnostic and therapeutic application. Diagnostic procedures include synovial fluid

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analysis and cytologic examination, to be performed especially in animals having joint effusion. The biomarkers that can potentially be used to detect different types of arthritis like osteoarthritis, septic arthritis are metabolic parameters *i.e.,* pH, glucose and lactate (Bakker *et al.,* 2021). The advantage of these metabolic biomarkers is that, their analytical methods are simpler and cheaper compared to the immuno-assays for the pro-inflammatory and degenerative biomarkers. Differential cell count is the sole method used to differentiate inflammatory arthropathies with non-inflammatory arthritis. Adjudging the importance of evaluating synovial fluid health, the present work was planned to describe their physical, biochemical and cytological characteristics in healthy young and adult dogs as possible standard reference values.

MATERIALS AND METHODS

Study was conducted on twelve non-descript dog carcasses. The dogs irrespective of sex were divided into two groups (6 dogs in each group) *i.e.,* group I (2-8 months of age) and group II (1-8 years of age). The work was performed at Department of Veterinary Anatomy, College of Veterinary Science and Animal Husbandry, NDVSU, Jabalpur for the duration of one year, *i.e.,* from September 2021 to August 2022. The dogs having healthy gait and joints as assessed by X-ray and ultrasonography (data not shown) died due to other non-related causes in the OPD of Veterinary Clinical Complex, Jabalpur were acquired with the consent of pet parents. Synovial fluid was collected aseptically by arthrocentesis of stifle joint following the standard protocol (Martinez and Santangelo, 2017). Immediately after collection, the volume and colour were recorded, and then transferred to a dry, sterile microcentrifuge tube without any anticoagulant. Smears of synovial fluid were made using the same techniques employed for blood. When the fluid was very viscous, the spreader slide was held at a low angle to spread the fluid over a larger area or spreader slide was placed upon the slide having the fluid and then both slides were pulled apart by sliding in opposite direction. After air drying, the smears were stained by Leishman / Wright stain (Drury and Wallington, 1980). The pH, viscosity, mucin quality, specific gravity and refractive index were measured. Protein and glucose were estimated within one hour using biochemical semi-autoanalyzer (Star 21 plus) (Table 1).

Statistical analysis

Statistical analysis was done with the help of R software (R team 2013) using independent 't' test and applied descriptive statistics ($p<0.05$).

RESULTS AND DISCUSSION

Volume of fluid collected in a single attempt varied from one drop to 0.5 ml. The mean volume of synovial fluid collected was 0.31±0.07 ml and 0.19±0.03 ml in group I and II respectively. Martinez and Santangelo (2017) stated that the volume that can be aspirated depends on the joint being sampled and the condition of the joint; in large breed dogs, less than 0.5 ml of fluid expected from most of the joints and up to 1 ml of fluid can be collected from stifle joint.

Table 1: List of parameters and methodology for analysis of synovial fluid.

Parameter	Description of methodology
Colour	Gross appearance of joint fluid and the interpretation of changes in appearance was made immediately after collection and graded as: • Clear and colourless- Normal. • Red or red-tinged- Indicates hemorrhage. • Yellow-orange discolouration- Indicates prior hemorrhage . • Turbid- Suspended particulates.
pH and specific gravity	Urine analyzer (Uri plus 200) (Sonawa *et al*., 2010).
Viscosity	Viscosity was assessed at the time of collection by visual observation using string test in which a drop of synovial fluid from a syringe was released over a slide and pulled away to produce a strand of at least 1 inch long before it broke (Fernandes, 2020).
Mucin quality	Mucin clot test: one part of synovial fluid was added to four parts of 2.5% glacial acetic acid in a microcentrifuge tube. The precipitate formed graded as: • Good - Tight ropy clot in a clear solution. • Fair - Soft clot with a turbid solution. • Poor - Friable clot in a cloudy solution. • Very poor - Flocculent material in a cloudy solution (Fernandes, 2020).
Refractive index	Firstly, brix values were measured with the help of MA871 refractometer. These values were interpreted using a table of ICUMSA (International Committee of Uniform Method of Sugar Analysis held in 1974) which represented refractive index values for Brix 0 to 85%.
Total protein (g/dl) and glucose (mg/dl)	Biochemical semi-autoanalyzer (Star 21 plus).

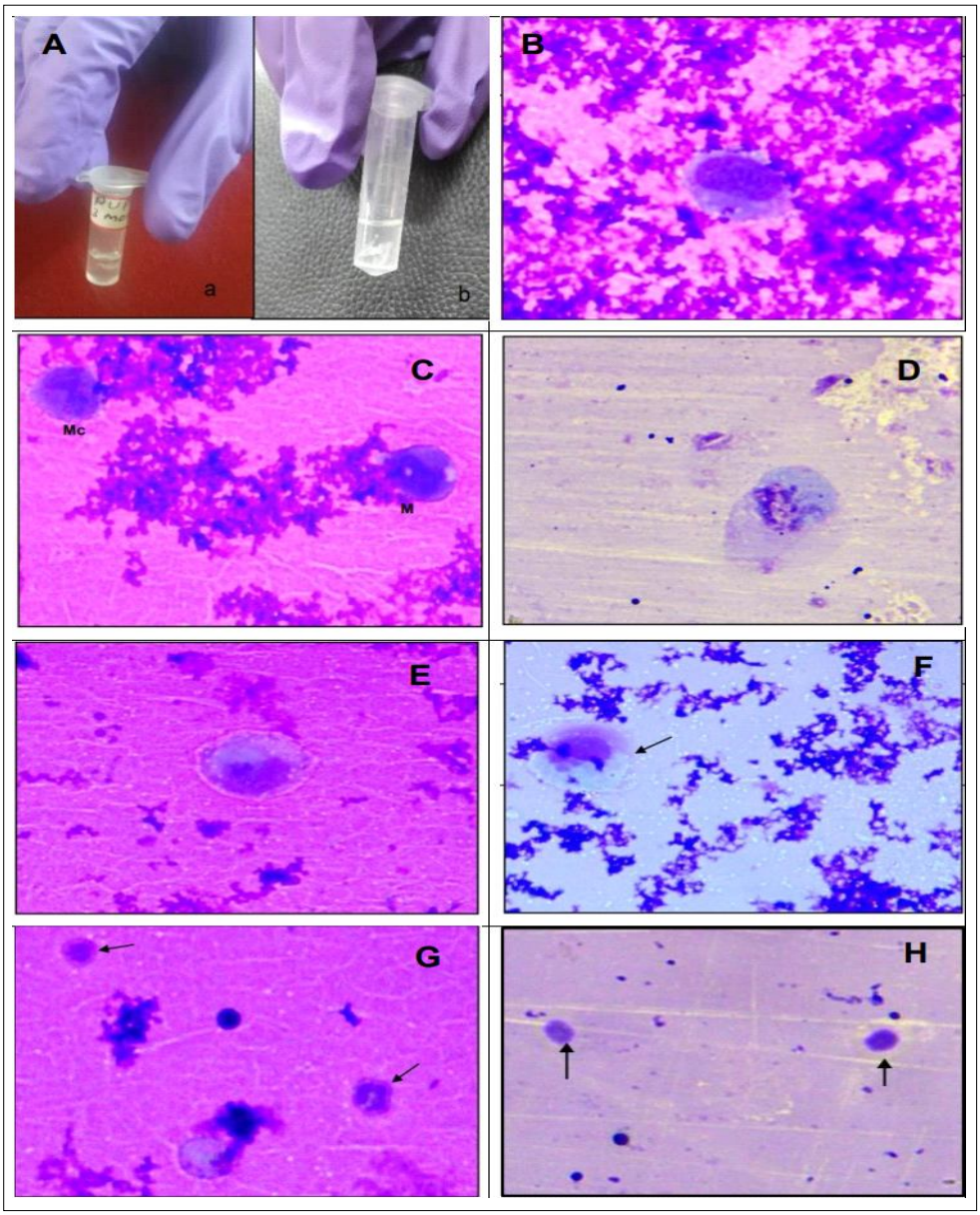

Fig 1: (A) Mucin clot test of synovial fluid. (a) Delicate clot in group I (b) Tight ropy clot in group II; B-H, photomicrograph of synovial fluid smear, Leishman stain, 1000x- (B) pink to purple granular background, monocyte with bean shaped nucleus, group II; (C) monocyte (M) and macrophage (Mc) group I; (D) synoviocyte with an eccentric nucleus and granulated cytoplasm, group I; (E) activated macrophage with vacuolated cytoplasm group II; (F) macrophage with oval nucleus, Wright stain (G) neutrophil (↑) having lobed nucleus, group I; (H) lymphocytes (↑) group I.

Table 2: Mean ± SE of physicochemical parameters of synovial fluid.

Parameter	Group I	Group II
pH	7.33±0.17	7.67±0.25
Refractive index	1.34±0.00	1.34±0.00
Specific gravity	1.02±0.00	1.02±0.00
Protein (g/dl)	1.29±0.18	2.74±0.84
Glucose (mg/dl)	102.79±3.66	105.47±4.19

The variations in the values of different parameters of synovial fluid within the group and between groups were statistically non-significant (P≤0.05)

Synovial fluid was clear and colourless in all samples of group I and II and did not clot in tube even after months of storage at 4°C. MacWilliams and Friedrichs (2003) reported that normal joint fluid is clear, colourless and does not clot in a tube or syringe but does tend to form a gel, which is in agreement with present findings. In contrast to present findings, Martinez and Santangelo (2017) described that normal synovial fluid in dogs is light yellow. Absence of spontaneous clot formation indicates that normal synovial fluid does not contain fibrinogen and other macromolecules of plasma.

There was no significant difference in mean pH values in group I (7.33±0.17) and II (7.67±0.25), similar pH range, varied from 7.0 to 8.0 was recorded in normal adult dog by Bakker *et al.* (2021).

Viscosity in all samples was found to be good as measured by string test. It showed a strand of more than 2.5 cm in both the groups. Cornelius (1963) stated that the viscosity of synovial fluid depends on amount and polymerization of hyaluronic acid. In inflammatory arthropathies hyaluronic acid degraded by proteases released by neutrophils results in decreased hyaluronic acid concentration (Fernandes, 2020). In the condition of septic arthritis, bacterial enzymes degrade the hyaluronate and decreased the viscosity. Value of refractive index and specific gravity of synovial fluid were non-significant in between and within the groups. Mean values of refractive index and specific gravity were 1.34 and 1.02, respectively in both the groups.

Observations of mucin clot test of synovial fluid in group I exhibited a tight ropy clot in a clear solution in samples of four animals however in two animals it showed a delicate clot with clear solution. All samples of group II exhibited a tight ropy clot in a clear solution. Turbidity was not observed in any sample. Thus, in both the groups there was presence of good quality mucin (Fig 1A). Cornelius (1963) stated that lubricating synovial mucin is an acid-glycoprotein, which precipitated by addition of common protein precipitants.

Mean protein concentration in group II was higher (2.74±0.84 g/dl) than group I (1.29±0.18 g/dl). The higher mean protein in group II might be due to age effect on permeability of synovial membrane.

Mean glucose concentration did not differ significantly in group I and II (Table 2). The values of glucose noted were in and around normal blood plasma level, thus it can be considered as dialysate of blood plasma, but low total protein values in synovial fluid might be due to reduced filtration of protein molecules through synovial membrane because of its larger molecular size.

Smears of synovial fluid under low power magnification showed background staining which appeared pinkish to purple, stringy, granular or clumped (Fig 1 B and C). This background staining was more pronounced in adult samples. MacWilliams and Friedrichs (2003) and Fernandes (2020) reported that the hyaluronic acid (mucin) content in normal synovial fluid causes the background staining to appear pink and fine to coarsely granular and to contain numerous crescents or folds. Decreased or increased density of granular eosinophilic background suggests its direct correlation with mucin content in joint fluid, thus a microscopic indicator of viscosity. The background material causes many of the nucleated cells to appear small, darkly stained, and difficult to recognize. In smears of some of the samples very few erythrocytes were observed. The body of the smear contained 1-3 cells per field at 1000x magnification. Synoviocytes were identified as round to oval cells having an irregular cytoplasmic membrane with abundant basophilic cytoplasm containing granules and an eccentrically placed nucleus (Fig 1D). There was variation in shape and size of these cells. Monocytes appeared round shaped cells with kidney shaped or indented nucleus (Fig 1B). Activated macrophages with vacuolated cytoplasm and ovoid eccentric nucleus were observed (Fig 1E). Inactivated macrophages identified as large cell with round to monocytoid nucleus without much vacuolation (Fig 1C and F). There

Table 3: Mean ± SE of differential cell count (%) in synovial fluid.

Cell type	Group I	Group II
Neutrophil	06.67±0.67	06.00±0.58
Small mononuclear cell	19.00±3.79	12.17±2.33
Large mononuclear cell	74.33±4.24	81.83±2.54

The variations in the values of different parameters of synovial fluid within the group and between groups were statistically non-significant (P≤0.05).

Table 4: Different types (%) of large mononuclear cells in synovial fluid.

Cell type	Group I	Group II
Monocyte	40.17±0.62	35.50±0.65
Macrophage	44.83±0.62	49.50±0.65
Synoviocyte	4.17±0.31	05.17±0.17
Large vacuolated macrophage	6.50±0.26	06.00±0.22
Irregular cell	4.00±0.17	03.17±0.37

The variations in the values of different parameters of synovial fluid within the group and between groups were statistically non-significant (P≤0.05).

were some irregular cells having granules in their cytoplasm with no defined morphology. Frequency of neutrophils was least among all cell types and comprised of multilobed nucleus (Fig 1G). For total cell count, cells were classified as small and large mononuclear cells and neutrophils (Fernandes, 2020). Large mononuclear cells included monocyte, macrophage, synovial lining cells and irregular cells. Small mononuclear cells included lymphocytes (Fig 1H) and small quiescent synoviocytes. Nucleated differential cell count was reported as percentage values (Table 3). Mean neutrophil count in both the groups was about 6% of total nucleated cells. Glyde (2018) mentioned that elevation of the relative proportion or absolute number of neutrophils (>10%) in synovial fluid indicates either inflammation of the synovial membrane or contamination with peripheral blood. Large mononuclear cells were found to be approximately 3/4th of total nucleated cell count in both the groups. Berg *et al.* (2009) reported differential count in stifle joint of dog and found 10-12% neutrophil, <30% small mononuclear cells and 60-97% large mononuclear cells. Curtiss (1964) reported rich existence of macrophagic cells in normal synovial fluid due to active migration of type A cells in joint cavity. Morphology of macrophages was assessed for increased cytoplasmic volume- with or without increased cytoplasmic vacuolation and foaminess. It was found that vacuolated large mononuclear cells contribute about 6-6.5% of total large mononuclear cells (Table 4). Berg *et al.* (2009) mentioned that <20% vacuolated macrophages in synovial smear could be considered as normal.

CONCLUSION

No significant difference was observed in most of physical and biochemical parameters of synovial fluid in young and adult dogs, however concentration of protein was higher in adult dogs. The baseline data may be helpful in differentiating between young and adult dogs and can be of referral value in diagnosing different arthropathies of the dog.

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